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Non-Invasive Imaging of F-Actin Dynamics in Living Cells by Atomic Force Microscopy
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Active and dynamic regulation of actin filaments (F-actin) is a key process in both constitutive and adaptive maintenance of cellular activities. We utilized a high-speed atomic force microscope (AFM) combined with an optical microscopy system, to visualize the dynamics of sub-membrane actin networks with sub-second time scale. Direct AFM imaging of a living COS-7 cell surface in growth medium revealed a cell-surface topography and membrane dynamics including endo/exocytosis. Increasing the scanning force enabled visualization of individual F-actin fibers constituting cortical actin networks. These filaments formed a three-dimensional meshwork with sizes of 1.7-14.0×104 nm2, which is in good agreement with the previous observations by super-resolution microscopy. Sub-second imaging of these actin networks over time revealed various dynamic rearrangements of the actin fibers and movements of actin-bound complexes. Fast polymerization of actin fibers was observed at a rate of >0.25 μm/sec, which is significantly faster than in vitro analyses (< 0.10 μm/sec). We occasionally observed actin-bound particles with various shapes (globule and rod) and sizes (100-500 nm along the long axis) moving along the fiber. Detailed analysis of the movement revealed that they moved in one direction along the fiber intermittently with an average velocity ranging 16-30 nm/sec. This is the first study which visualizes dynamics of single F-actin fibers with their surrounding environment in a live cell with high temporal and spatial resolution, and provides a novel non-invasive technique to investigate F-actin dynamics in a live cell.

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Studying the Mechanical Properties of Cytoskeletal Formation using Microrheology
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During the first stages of embryogenesis in zebrafish, the shape and size of the zygotic cell change dramatically due to the forming cytoskeleton. As the microtubules extend outward from the center of the cell they push the centrally located filamentous actin towards the cortex. This incredible morphological change reorganizes the cellular components, including organelles and germ plasm RNA. Yet, the mechanical properties of this rearrangement are unknown. Here, we use particle tracking microrheology to measure the viscoelastic properties of the zebrafish embryo as the cytoskeleton forms. To do so, we inject fluorescent beads into the one-cell stage of the embryo and record the beads' movement until cellular cleavage. We then use this motion to calculate the mean squared displacement (MSD) of the beads and the viscoelastic properties of the cytoplasm. We find that the cell is apparently viscous with an average viscosity of 0.04 Pa·s, which is about 40 times that of water and approximately 2 orders of magnitude less than a C. elegans embryo. Interestingly, we also see a radial viscosity increase that is coincident with the movement of filamentous actin towards the cortex.

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Adaptation of Actin Cytoskeleton during Suspended Animation
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Confronted with limited oxygen, numerous organisms demonstrate evolutionarily conserved responses, arresting fundamental biological processes to achieve a physiological state termed suspended animation. An example of direct relevance to human biology and disease is the vertebrate zebrafish, Danio rerio. Physiologically active embryos with heartbeat, circulation, and a functional central nervous system arrest all of these dynamic processes, enter a state of suspended animation during anoxic exposure and then recover with normal developmental program upon return to normoxia. The same response occurs with inhibitors of the oxidative phosphorylation, indicating that inhibition of the mitochondrial production of ATP, rather than absence of molecular oxygen, is the proximate signal inducing suspended animation. Although this unique defense strategy has been extensively studied for more than a century, the biophysical mechanisms remain largely unknown. Our proteomic analysis during the first hour of anoxia implicated rapid regulation of the actin cytoskeleton.
We have now discovered a rapid and reversible adaptation of actin cytoskeleton during anoxia. Using fluorescent labels that target monomeric and filamentous actin, we have quantified changes in the distribution of muscle fibers in somites, an increase in the abundance of monomeric actin in pronephric duct, and thickening of filamentous actin bundles at the cortex of keratinocytes. Consistent with these observations, we observe rapid arrest in growth of muscle fibers during anoxia and rapid recovery during normoxia. The time course of these events suggests that suspended animation requires rapid and dynamic adaptation of the existing cytoskeleton and our data suggest that this process may be critical to tissue preservation and survival.

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How Does the Interplay between Semiflexible Polymers Determine Composite Network Mechanics?
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The semiflexible polymers actin and intermediate filaments (IF) intertwine in a complex network within the cell, and together are key determinants of cellular stiffness. While the mechanics of actin networks together with stiff microtubules have been characterized, the interplay between actin and IF networks is largely unknown, necessitating the study of composite networks using mixtures of semiflexible biopolymers. We employ bulk rheology in a simplified in vitro system to uncover the fundamental mechanical interactions between networks of the two semiflexible polymers, actin and vimentin IF. Surprisingly, co polymerization of actin and vimentin can produce composite networks either stronger or weaker than pure actin networks. We show that this effect occurs through steric constraints imposed by IF on actin during network formation and filament crosslinking, highlighting novel emergent behavior in composite semiflexible networks.
In addition, we find that vimentin IF have little effect on actin network mechanics when actin is crosslinked with alpha-actinin. However, when using Filamin-A as an actin crosslinker, co-polymerization with vimentin creates a stiffer network able to better maintain its elasticity under larger strains, but with a lower yield stress.

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Axial Elasticity and Mechanical Fragmentation of Desmin Intermediate Filaments
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Desmin is the intermediate filament of muscle cells. Desmin filaments are thought to play fundamental roles in cellular force transduction and the maintenance of structural integrity under mechanical exposure. Although the importance of desmin elasticity and assembly/disassembly dynamics in cellular mechanics is being increasingly recognized, the molecular basis of desmin's elasticity or its disassembly pathway are little understood. In the present work we explored the elasticity of purified desmin filaments by stretching them longitudinally with surface-tension forces.
Desmin, purified from chicken gizzard, was polymerized by the addition of MgCl2. Polymerized filaments in a buffer droplet were then stretched by using a custom-built horizontal rotor assembly. Driven by centrifugal force, the droplet spread radially on freshly cleaved mica surface. Partially surface-adsorbed desmin filaments became extended by the receding meniscus and were captured on mica in the over-stretched state. The stretch force acting on the entire cross-sectional area of desmin was calculated to be 4 nN.
As a result of this molecular combing, the average contour length of desmin filaments increased from 0.89 μm to 1.38 μm, which corresponds to a 1.6-fold axial stretch. Molecular combing together with EDTA-treatment caused the fragmentation of desmin filaments into short, 60- to 120-nm-long and 4-nm-wide structures displaying periodic, 34 nm-surface protrusions. Based on these calculations the observed fragments are hypothesized to be protofibril fragments composed of laterally attached desmin dimers. The orientation axis of the surface-constrained fragments deviated widely from the filament axis, suggesting that the protofibrils are not aligned in parallel within the filament during axial load. Furthermore, the emergence of protofibril fragments suggests that the head or tail domains of coiled-coil desmin dimers are the load-bearing elements during axial stretch.

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A Compete-And-Survive Mechanism Explains the Single FtsZ-Ring Formation
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Cytokinesis is a critical step in cell reproduction. In prokaryotic cells, such as Escherichia coli, this process is mediated by a cytoskeletal structure (i.e., Z ring), which is assembled from FtsZ protofilaments that are ''anchored'' to the cell membrane through ZipA/FtsA molecules, and serves as the scaffold